

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	ImageJ, ObjectJ, Fil-Tracer, MicroBJ 5.13J python v3.7.12 Matlab 2015 R v4.0.3 or v3.6.1 samtools v1.9 sortmerna v4.1.0 guppy 5.0.11+2b6dbffa5
Data analysis	genome assembly: canu v2.1.1, racon v1.4.3, minimap2 v2.7, medaka v1.74.0, pilon v1.22, bwa v0.7.16a, CheckM v1.0.18, Prokka v1.14.6, eggnoG mapper v2.1.10 ParB phylogeny: mafft v7.427, IQ-TREE v2.1.2, FigTree v1.4.4 ori and ter determination: gplots v3.1.1 in R 3.6.1, bowtie2 v2.4.4, samtools v1.9, deeptools v3.5.1, UCSC v391, circos v0.69-8 88 in Perl 5.028003 MFA: trimmomatic v0.39, prinseq-lite v0.20.4, bbmap v38.90, bowtie2 v2.4.4, samtools v1.9, deeptools v3.5.1, UCSC v391, ggplot2 64 in R v3.6.1 3C: https://github.com/axelcournac/EColi_analysis , custom code for FI and loop analysis, Matlab 2015, normalization by Iterative correction and eigenvector decomposition (ICE) RNA-Seq: trimmomatic v0.39, prinseq-lite v0.20.4, sortmerna v4.1.0, bowtie2 v2.4.4, subread-package v2.0.0, bowtie2 v2.4.4, samtools v1.9,, python v3.7.12, DESeq2 package v1.30.1, R v4.0.3

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Data generated in this study have been deposited in the NCBI repository. Specifically, data for genome sequencing has been deposited in Bioproject PRJNA687213 (A. filiformis), PRJNA687218 (S. muelleri), and PRJNA779076 (C. steedae). Genomes have been deposited in GenBank as GCF_020162295.1 (A. filiformis), GCF_020162275.1 (S. muelleri), and GCF_023547005.1 (C. steedae). Data for marker frequency analysis has been deposited in Bioproject PRJNA795449 (A. filiformis), PRJNA795451 (S. muelleri), and PRJNA795450 (C. steedae), and corresponding non-normalized, 1kb-binned read count files in Gene Expression Omnibus (GEO) GSE194131 (A. filiformis), GSE194134 (S. muelleri), and GSE205139 (C. steedae). Data for RNA-Seq has been deposited in Bioproject PRJNA795348 (A. filiformis) and PRJNA795350 (S. muelleri), and corresponding raw read count files in GEO GSE194132 (A. filiformis), and GSE194135 (S. muelleri). Data for 3C-seq has been deposited in Bioproject PRJNA837061 (A. filiformis), PRJNA837062 (S. muelleri), PRJNA1052629 (C. steedae), and corresponding matrices in GEO GSE250400 (A. filiformis), GSE250402 (S. muelleri), and GSE250401 (C. steedae). See also Supplementary Table 4 for SRA accession codes.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\)](#), [and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For RNA-seq analyses, 3 independent biological replicates were carried out for each studied symbiont. The statistical significance of the RNA-seq data was assessed using a DESeq2-based pipeline. For 3C-seq analysis, we used 10 to the 8 cells per experiment. For DNA staining, DNA FISH and immunostaining the number of analyzed cells has been included to the Figures.
Data exclusions	No data exclusion
Replication	3C-seq is a bulk assay which pool millions of cells (typically 10 to the 8 per experiment), creating an internal "pseudo-replication" of homogenous nuclear states that stabilizes global patterns (also referred to as population averaging). However, for 3C-seq analysis of planktonic exponential phase A. filiformis, two independent experiments (performed in different days) were carried out. DNA FISH, ParB immunostaining, EMSAs were repeated at least three times each on different days. We kept the same procedures and same standards for sample collection and analysis. ParB and Ig (control) ChIP-seq was each performed in triplicates on the same day.
Randomization	Sample collection and measurements were performed independently (on different days, with different medium preparation) in order to minimize experimental bias. Our experiments did not involved allocation into experimental groups.
Blinding	Blinding is not relevant for this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	The primary antibody used for <i>Ca. T. hypermnestrae</i> was a peptide anti-Thiosymbion ParB antibody (Weber et al., 2019). The antibody used for the multicellular Neisseriaceae was a guinea pig polyclonal antibody against recombinant <i>S. muelleri</i> ParB (1:1000 dilution). For Western blots, the secondary antibody used was a horseradish peroxidase-conjugated anti-rabbit or anti-guinea pig secondary antibody (Amersham Biosciences) with a 1:10,000 dilution. For immunostaining, we used a 1:500 dilution of secondary Alexa 488-conjugated anti-rabbit antibody.
Validation	We validated the antibody with Western-blot against purified ParB and bacterial protein extracts.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

ChIP-seq

Data deposition

- ☒ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication.</i>	NCBI GEO reviewer access token gtmfauwozfojncf (A. filiformis), sbavygampetlab (S. muelleri)
Files in database submission	GSE322725_af_bamcompare.bw (A. filiformis), GSE322727_sm_bamcompare.bw (S. muelleri)
Genome browser session (e.g. UCSC)	NCBI Refseq: GCF_020162295.1 (A. filiformis), GCF_020162275 (S. muelleri)

Methodology

Replicates	For each organism, 3 biological replicates (3 whole cell extracts) for anti-ParB antibody and 3 biological replicates (3 whole cell extracts) for control
Sequencing depth	Total number of reads: 11148355, 9739618, 10018146 (anti-ParB); 11068849, 10131780, 10459953 (control) (A. filiformis); 7823063, 8209056, 9811224 (anti-ParB) (control); 8204028, 2162991, 8022346 (S. muelleri); Uniquely mapped reads: 9790168, 8696547, 8933957 (anti-ParB); 10163370, 9077594, 9649211 (control) (A. filiformis); 6595254, 6787791, 8081664 (anti-ParB) (control); 6409991, 928195, 6127182 (S. muelleri);

	Length: 100 bp, single-end reads
Antibodies	Custom guinea pig polyclonal antibody against recombinant <i>S. muelleri</i> ParB (not commercially available)
Peak calling parameters	Mapping: Bowtie2 v2.4.4 with default parameters; no peak calling was performed.
Data quality	Not available as peak calling was not performed.
Software	bamCompare from deepTools v3.5.1, with log2 ratio transformation, BPM normalization, bin size 10 bp, pseudocount of 1, and without scaling factor correction; plotted with R v.3.6.1